Sensing Log Book (June)

MONDAY, 6/12/2017

AIm: Transformation of pSB1C3-BBa_c0051 and pSB1C3-BBa_T9002

TUESDAY, 6/13/2017

1. DNA extraction from kit plate
    - pSB1C3-BBa_C0051 (2014 Spring Distrubution kit, plate 2, well 4B)
    - pSB1C3-BBa_T9002 (2013 Spring Disribution kit, plate 4, 21I)

2. Transformation of pSB1C3-BBa_C0051, pSB1C3-BBa_T9002 and pSB1A2-BBa_C0261

Table1

	A	B
1	Reagent	Volume (µl)
2	Competent cell	50
3	DNA template	1
4	Total volume	51

Table 1. Reagent of Transformation

3. Spread plate
    - 20µl of pSB1C3-BBa_C0051
    - 200µl of pSB1C3-BBa_C0051
    - 20µl of pSB1C3-BBa_T9002
    - 200µl of pSB1C3-BBa_T9002
    - 50µl of pSB1A2-BBa_C0261

WEDNESDAY, 6/14/2017

Result from (12/6) transformation plate:
Spread plate

- pSB1A2-BBa_C0261: 2 colonies
- pSB1C3-BBa_C0051: 4 colonies
- pSB1C3-BBa_T9002: no colonies

1. Streaking of pSB1A2-BBa_C0261 and pSB1C3-BBa_C0051

2. Second transformation of pSB1C3-BBa_T9002 (From 2014 kit plate)

Table2

	A	B
1	Reagent	Volume (µl)
2	Copetent cell	50
3	DNA template	1
4	Total volume	51

Table 2. Reagent of Transformation

Protocal changed for the 2 transformation sample:
Invitrogen protocol: 200µl LB for recovery, 45s for heat shock, 2 mins for cold shock
iGEM protocol: 200µl LB for recovery, 1 min for heat shock, 5 mins for cold shock

3. Spread plate

- 200µll of sample pSB1C3-BBa_T9002 (from 12/6) for 1 hour of 2nd recovery
- 200µll of pSB1C3-Bba_T9002 (with 2 different protocol)

Total: 3 plates

13-6-17_pSB1A2-BBa_C0261_1_.jpg

THURSDAY, 6/15/2017

Results from (13/6) transformation plate:

Spread plate:

- pSB1C3-BBa_T9002 (Second transformation) : no colonies

1. Inoculation of pSB1A2-BBa_C0261 and pSB1C3-BBa_C0051
2. Third transformation from Kit plate of psB1C3-BBa_T9002 (2014)

Table3

	A	B
1	Reagent	Volume (μl)
2	Copetent cell	50
3	DNA template	1
4	Total volume	51

Table 3. Reagent of Transformation

Summer lab training protocol: 1ml LB for recovery, centrifuge at 7000 rpm for 2.5 mins, 45s for heat shock, 2 mins for cold shock
iGEM protocol: 200μl LB for recovery, 1 min for heat shock, 5 mins for cold shock

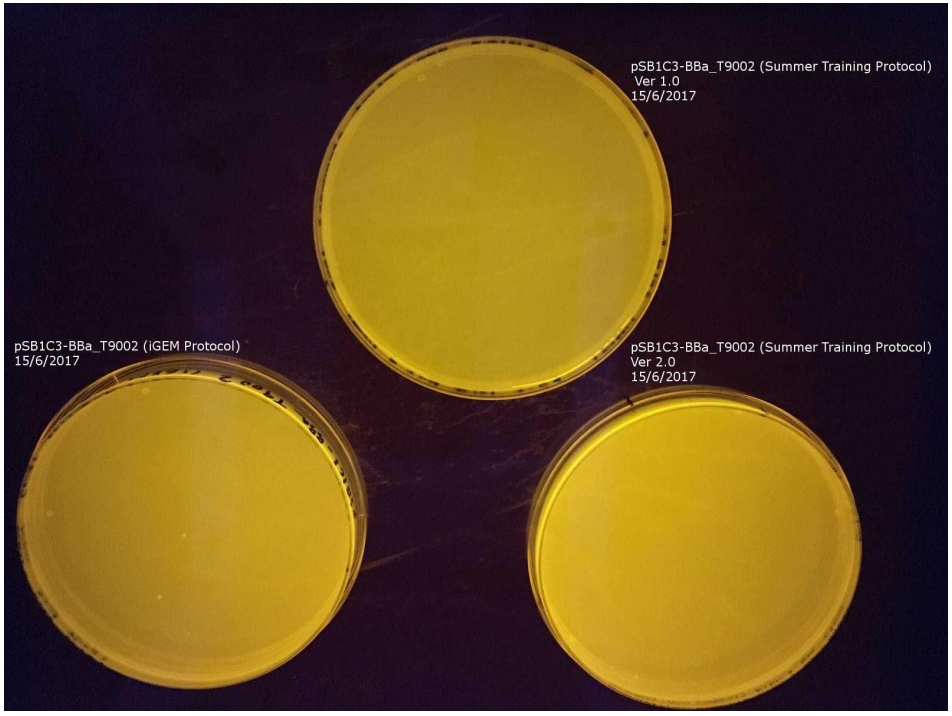
3. Spread plate
   - 200μll of pSB1C3-Bba_T9002 (Summer training protocol) iincubated on ice for 20 minutes before recovery
   - 200μll of pSB1C3-Bba_T9002 (with 2 different protocol) for 1 and a half hour of recovery

Total: 2 plates

FRIDAY, 6/16/2017

Result:
pSB1C3-BBa_T9002 (iGEM protocol): 3 colonies
pSB1C3-BBa_T9002 (Summer lab training) Ver 1.0: 0 colonies
pSB1C3-BBa_T9002 (Summer lab training) Ver 2.0: 0 colonies

15-6-17_pSB1C3-BBa_T9002_diff_protocol_.jpg



1. Streak plate of pSB1C3-BBa_T9002 (iGEM protocol)
2. Miniprep of inoculated pSB1C3-BBa_C0051 and pSB1C3-BBa_C0261

Table4

	A	B	C
1		pSB1C3-BBa_C0261	pSB1C3-BBa_C0051
2	Protein Contamination	1.807	1.848
3	Salt Contamination	1.548	2.043
4	DNA Concentration	64.94 µg/mL	542.5 µg/mL**

Table 4. Result of Miniprep of inoculated pSB1C3-BBa_C0051 and pSB1C3-BBa_C0261

(**abnormal concentration, contamination of host DNA due to miniprep error)

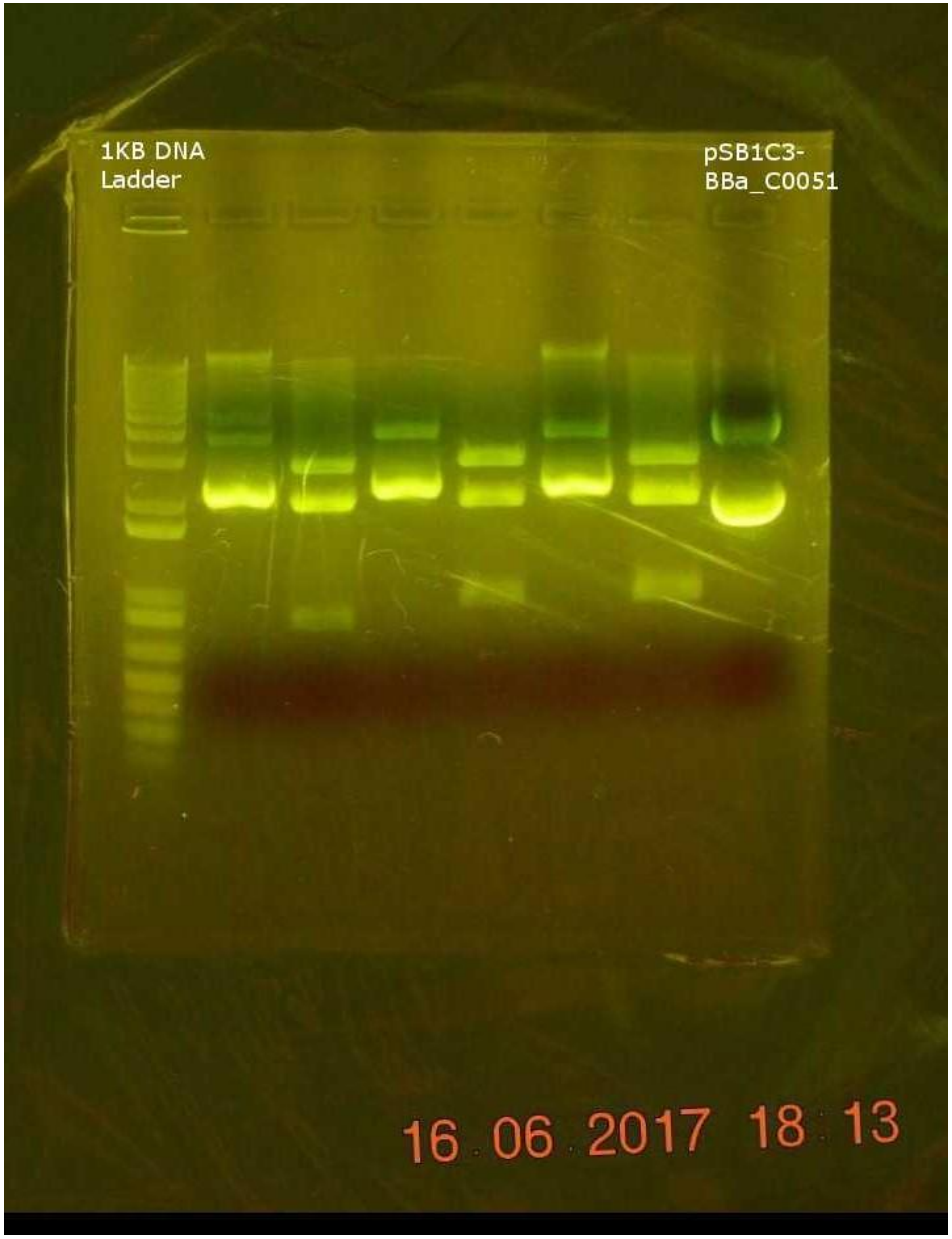
3. Inoculation of pSB1C3-BBa_C0051 overnight
4. Plate streaking of pSB1C3-BBa_C0051 (second streak)

SATURDAY, 6/17/2017

Result form (15/6): successful streaking of pSB1C3-BBa_T9002 and pBS1C3-BBa_C0051

1. Storage of streaked plates into fridge
    - pSB1C3-BBa_T9002 (iGEM protocol)
    - pSB1C3-BBa_C0051 (second streak)
2. Miniprep of pSB1C3-BBa_C0051
   Result :
   Protein contamination: 1.849
   Salt contamination: 2.332
   DNA concentration: 411.5 µg/mL
3. Gel-electtrophoresis: contamination of host DNA

19-6-17_pSB1C3-BBa_F2620_pSB1A2-BBa_C0261_1_.jpg



MONDAY, 6/19/2017

Group 1

1. DNA extention from kit plate
    - BBa_J61002-J23100 (2010 kit plate 1, 18C)
    - BBa_J61002-J23113 (2009 kit plate 1, 20G)
    - BBa_J61002-J23115 (2009 kit plate 1, 20K)
2. Inoculation + Spread plate
    - BBa_J61002-J23100
    - BBa_J61002-J23113
    - BBa_J61002-J23115
    - BBa_J61002-J23110
    - BBa_J61002-J23117

Group 2

1. Digestion

Table5

	A	B
1	Reagent	Volume (μL)
2	S	0.2
3	P	0.2
4	DNA	15.8
5	cutsmart buffer	1.8
6	ddH20	0.4

Table 5. Digestion of pSB1C3-BBa_F2620

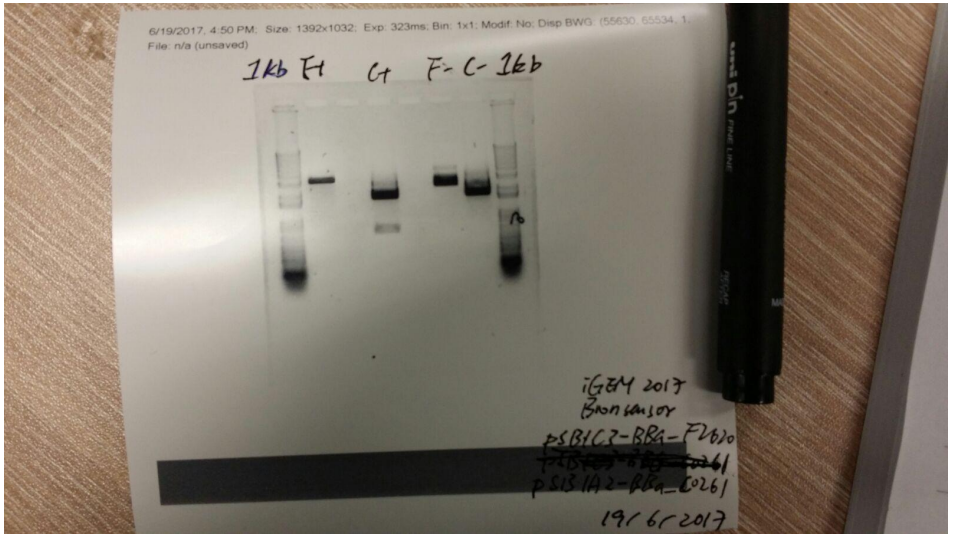
Table6

	A	B
1	Reagent	Volume (µL)
2	P	0.2
3	X	0.2
4	DNA	14.7
5	cutsmart buffer	1.8
6	ddH20	1.6

Table 6. Digestion of pSB1A2-BBa_C0261

2. Inoculation of pSB1C3-BBa_F2620 and pSB1A2-BBa_C0261 using CHL adn AMP LB
3. Gel eletrophoresis using 1% gel (0.2g agarose, 0.2µL midorigreen, 20mL buffer)
4. Gel photo and gel cutting

19-6-17 pSB1C3-BBa_F2620 pSB1A2-BBa_C0261 (2).jpeg



TUESDAY, 6/20/2017

Group 1:

1. Inoculation and streak plate of J23100, J23117
2. 2nd transformation of J23110, J23113, J23115 due to no colonies

Group 2:

1. Gel purification of pSB1C3-BBa_F2620 and BBa_C0261

Table7

	A	B	C
1		pSB1C3-BBa_F2620 (digested)	BBa_C0261 (digested)
2	Protein Contamination	0.007	0.177
3	Salt Contamination	2.866	1.790
4	DNA Concentration	9.216 μg/mL	4.53 μg/mL

Result of Gel purification of pSB1C3-BBa_F2620 adn BBa_C0261

2. Ligation of digested product

Table8

	A	B	C
1	Reagent	Volume (μl)	neagtive control (μl)
2	T4 ligase	0.5	0
3	ligase buffer	1	1
4	backbone	3.62	3.62
5	insert	4.88	4.88
6	MQ	0	0.5
7	Total volume	10	10

Reagent

3. Inoculation of ligated product + spread plate

Group 3:

1. Miniprep of pSB1C3-BBa_F2620 and pSB1A2-BBa_C0261

Table9

	A	B	C
1		pSB1C3-BBa_F2620	pSB1A2-BBa_C0261
2	DNA concentration	1161.1μg/mL	86.96μg/mL
3	Protein Contamination	2,272	2.024
4	Salt Contamination	1.840	1.852

Result of Miniprep of pSB1C3-BBa_F2620 and pSB1A2-BBa_C0261

WEDNESDAY, 6/21/2017

Group 1
Result: BBa_J61002-J23110: 2 colonies (red)
Streaking and inoculation of BBa_J61002-J23110

Group 2
Colony of PCR adn Gel electrophoresis

1. 5 white colonies sample from psB1C3-BBa_F2620-C0261
2. 1 sample from BBa_J61002-J23100 (20/6)
3. 1 sample from BBa_J61002-J23117 (20/6)

Group 3
Transformation and spread plate

1. BBa_J61002-J23100 (from prepared stock)
2. BBa_J61002-J23100 (newly extracted from 2011 kit plate)
3. BBa_J61002-J23117 (from prepared stock)
4. BBa_J61002-J23117 (newly extracted from 2011 kit plate)
5. BBa_J61002-J23113 (newly exctraced from 2011 kit plate)
6. BBa_J61002-J23115 (newly extrcated from 2011 kit plate)

Table10

	A	B	C
1	Reagent (µl)	Volume (µl)	Master Mix (X15)
2	MQ	12.875	193.125
3	5X my jaq	4	60
4	10µM VF2	0.5	7.5
5	10µM VR	0.5	7.5
6		0.125	1.875
7	Template	2	/
8	Total	20	/

MasterMix Solution

Table11

	A	B	C
1	Steps	Temperature (oC)	Time
2	initial denaturation	95	3 mins
3	denaturation	95	30s
4	annealing	55	1 min
5	extension	68	2 mins 15s
6	final extension	68	5 mins
7	holding	4	infinitive

Colony PCR

THURSDAY, 6/22/2017

Result

BBa_J61002-J23117 (from prepared stock): bacterial lawn (white)

BBa_J61002-J23100 (newly extracted from 2011 kit plate): many white colonies

BBa_J61002-J23117 (from prepared stock): 1 scolony (white)
BBa_J61002-J23117 (newly extracted from 2011 kit plate): no colony
BBa_J61002-J23113 (newly exctraced from 2011 kit plate): no colony
BBa_J61002-J23115 (newly extrcated from 2011 kit plate): no colony
Streaking and inoculation of BBa_J61002-J23110: have colonies

Group 1
Transformation and Spread plate

1. BBa_J61002-J23100 (2011 kit plate)
2. BBa_J61002-J23117 (2011 kit plate)
3. BBa_J61002-J23113 (2011 kit plate)
4. BBa_J61002-J23115 (2011 kit plate)

Group 2
Gel electrophoresis (pSB1C3-BBa_F2620-C0261 colony PCR)
Sample 1-5: pSB1C3-BBa_F2620-C0261
Sample 6: BBa_J61002-J23110
Sample 7: BBa_J61002-J23117
+: pSB1C3-BBa_T9002
-: pSB1C3-BBa_F2620 (digested)
w: water

Group 3
Miniprep of J23110

Table12

	A	B
1	DNA concentration	112.2μg/ml
2	Protein concentration	1.804
3	Salt concentration	1.695

Table 12: Result of Miniprep of J23110

FRIDAY, 6/23/2017

Result

BBa_J61002-J23100 and pSB1A2-BBa_J23117: bacterial lawn
BBa_J61002-J23113 and pSB1A2-BBa_J23115: no colony

Colony PCR of pSB1C3-BBa_F2620-C0261
Sample 1-5: pSB1C3-Ba_F2620
Sample 100: BBa_J61002-J23100
Sample 117: BBa_J61002-J23117
+: pSB1C3-BBa_T9002
-1: pSB1C3-BBa_F2620 (plasmid)
-2: pSB1C3-BBa_ F2620 (digested)
w: water

Mastermix preparation:

Table13

	A	B	C
1	Reagent (µl)	Volume (µl)	Master Mix (x15)
2	MQ	14.375	215.7
3	Thermal Pol	2	30
4	10mM dNTP	0.5	7.5
5	10mM VF2	0.5	7.5
6	10mM VR	0.5	7.5
7	Taq Polymerase	0.125	1.875
8	DNA Template	2	/
9	Total	20	/

MONDAY, 6/26/2017

Group 1: Digestion of pSB1A2-BBa_E0240 and pSB1A2-BBa_J23110

For pSB1A2-BBa_E0240:

Table14

	A	B	C
1	Component	Volume (μl)	Negative control
2	XbaI	0.2	/
3	PstI	0.2	/
4	Cutsmart	1.8	1.8
5	DNA (E0240)	4.47	4.47
6	ddH2O	11.33	11.73
7	Total	18	18

Concentration: 112.2 ng/μl
Mass: 500 ng
Expected band size: 902 & 2053 bp

For pSB1A2-BBa_J23110:

Table15

	A	B	C
1	Component	Volume (μl)	Negative control
2	S	0.2	/
3	XBaI	0.2	/
4	Cutsmart	1.8	1.8
5	DNA	4.456	4.456
6	ddH2O	11.34	11.74
7	Total	18	18

Concentration: 111.7 ng/μl
Mass: 500 ng
Expected band size: 2096 & 18 bp

Group 2: Transformation of J23100 (Newly extracted from kit plate)

Table16

	A	B
1	Reagent	Volume (µl)
2	DNA	1
3	Compentent cell	50
4	Total	51

Group 3: Inoculation of pSB1C3-BBa_F2620_C0261 from Sample 1 (from colony PCR) and pSB1A2-BBa_J23117

TUESDAY, 6/27/2017

Result: Transformation of J23110: 0 colonies

Group 1: Transformation of J23110

- 1 µl of plasmid J23110 (2012) into 50 µl compentant cell
- 1 µl of plasmid J23110 (2009,2010,2011) into 50 µl compentant cell

Group 2:

1. Miniprep:
    a. pSB1C3-BBa_F2620-C0261

Result:

Table17

	A	B
1	DNA Concentration	259.2
2	Protein Contamination	1.823
3	Salt Contamination	2.310

b. BBa_J61002-J23117

Result:

Table18

	A	B
1	DNA Concentration	72.98
2	Protein Contamination	1.739
3	Salt Contamination	0.973

2. Digestion

Table19

	A	B	C
1	Reagents	BBa_J61002-J23110	(-)
2	SpeI (μl)	0.2	/
3	Pst I (μl)	0.2	/
4	MQ (μl)	8.638	12.169
5	Cutsmart (μl)	1.8	1.8
6	DNA (μl)	7.162	3.581
7	Total (μl)	18	18

Table20

	A	B	C
1	Reagents	pSB1A2-BBa_E0240	(-)
2	Xball (μl)	0.2	/
3	Pst I (μl)	0.2	/
4	MQ (μl)	11.344	11.744
5	Cutsmart (μl)	1.8	1.8
6	DNA (μl)	4.456	4.456
7	Total (μl)	18	18

Table21

	A	B	C
1	Reagents	pSB1C3-BBa_F2620-C0261	(-)
2	SpeI (μl)	0.2	/
3	Pst I (μl)	0.2	/
4	MQ (μl)	12.71	14.7
5	Cutsmart (μl)	1.8	1.8
6	DNA (μl)	3.09	1.5
7	Total (μl)	18	18

Table22

	A	B	C
1	Reagents	BBa_J61002-J23117	(-)
2	SpeI (μl)	0.2	/
3	Pst I (μl)	0.2	/
4	MQ (μl)	4.84	11.2
5	Cutsmart (μl)	1.8	1.8
6	DNA (μl)	10.96	5.0
7	Total (μl)	18	18

3. Gel electrophoresis (0.8% agarose gel)
   - 110V, 30 minutes

4. Cut gel

WEDNESDAY, 6/28/2017

1. Streaking and inoculation of J23100

2. Gel purification
   - pSB1C3-BBa_F2620-C0261 (3782, 18)

Result:

Table23

	A	B
1	DNA Concentration	42.92
2	Protein Contamination	1.716
3	Salt Contamination	0.564

- BBa_J61002-J23110 (2096)

Result:

Table24

	A	B
1	DNA Concentration	10.21
2	Protein Contamination	1.960
3	Salt Contamination	0.505

- pSB1A2-BBa_E0240 (902)

Result:

Table25

	A	B
1	DNA Concentration	19.96
2	Protein Contamination	1.821
3	Salt Contamination	0.384

- BBa_J61002-J23117 (2096, 887)

Result:

Table26

	A	B
1	DNA Concentration	9.212
2	Protein Contamination	2.187
3	Salt Contamination	0.071

3. Ligation of J23110- E0240, F2620-C0261-E0240, J23110-E0240

F2620-C0261-E0240:

Table27

	A	B	C
1		F2620-C0261-E0240	(-)
2	Insert length (bp)	902	902
3	Backbone length (bp)	3782	3782
4	Insert Concentration (ng/μl)	19.96	19.96
5	Backbone Concentration (ng/μl)	47.92	47.92
6	Insert Volume (μl)	5.37	5.37
7	Backbone Volume (μl)	3.12	3.12
8	10x Buffer (μl)	1	1
9	Ligase (μl)	0.5	/
10	MQ (μl)	/	0.5
11	Total	10	10

For J23110-E0240 positive control, there are 2 samples:

- J23110-E0240 (1) : Incubate in room temperature for 10 minutes before transformation
- J23110-E0240 (2) : Incubate in room temeperture for 1 hour before transformation

Table28

	A	B	C	D
1		J23110-E0240 (1)	J23110-E0240 (2)	(-)
2	Insert length (bp)	902	902	902
3	Backbone length (bp)	2096	2096	2096
4	Insert Concentration (ng/μl)	19.96	19.96	19.96
5	Backbone Concentration (ng/μl)	10.21	10.21	10.21
6	Insert Volume (μl)	3.38	3.38	3.38
7	Backbone Volume (μl)	5.12	5.12	5.12
8	10x Buffer (μl)	1	1	1
9	Ligase (μl)	0.5	0.5	/
10	MQ (μl)	/	/	0.5
11	Total	10	10	10

J23117-E0240:

Table29

	A	B	C
1		J23117-E0240	(-)
2	Insert length (bp)	902	902
3	Backbone length (bp)	2096	2096
4	Insert Concentration (ng/μl)	19.96	19.96
5	Backbone Concentration (ng/μl)	9.212	9.212
6	Insert Volume (μl)	3.17	3.17
7	Backbone Volume (μl)	5.33	5.33
8	10x Buffer (μl)	1	1
9	Ligase (μl)	0.5	/
10	MQ (μl)	/	0.5
11	Total	10	10

4. Transformation and spreading

Table30

	A	B	C	D
1	Reagents	pSB1C3_BBa_F2620-C0261-E0240	pSB1A2_BBa_J23110-E0240	pSB1A2_BBa_J23117-E0240
2	DNA (μl)	10	1	10
3	Competent Cell (μl)	50	50	50
4	Total (μl)	60	51	60

THURSDAY, 6/29/2017

Result: Transformation of BBa_J61002-J23100: No colonies

Colony PCR: (1% Agarose, 30 minutes, 130 V)

Table33

	A	B	C
1		Samples	Expected band size
2	1-5	pSB1C3-BBa_F2620_C0261_E0240	2920bp
3	6-10	pSB1A2-BBa_J23110_E0240 (10 mins)	1149bp
4	11-15	pSB1A2-BBa_J23110_E0240 (1 hour)	1149bp
5	16-20	pSB1A2-J23117_E0240	1149bp
6	+ for pSB1A2-BBa_J23110_E0240	BBa_J61002_J23110	1142bp
7	(-) for pSB1C3-BBa_F2620_C0261_E0240	pSB1C3-BBa_F2620_C0261_E0240	2259bp

- Following the previous protocol mentioned from above

FRIDAY, 6/30/2017

Result: Failed colony PCR from 29/6

1. Colony PCR

Table34

	A	B
1		Sample
2	1-5	pSB1C3-BBa_F2620_C0261_E0240
3	6-10	pSB1A2-BBa_J23110_E0240 (10 mins)
4	11-15	pSB1A2-BBa_J23110_E0240 (1 hour)
5	16-20	pSB1A2-BBa_J23117_E0240
6	+ PCR control for pSB1A2-BBa_J23110-E0240	BBa_J61002_J23110
7	(-) for pSB1C3-BBa_F2620_C0261_E0240	pSB1C3-BBa_T9002

- Adopted a new protocol for colony PCR:

Table35

	A	B	C
1	Reagents	Volume (µl)	Master Mix (µl) x25
2	MQ	33	825
3	5x My Taq (Reaction buffer)	10	250
4	10µM VF2	2	50
5	10µM VR	2	50
6	Taq polymerase	1	25
7	Template	2	/
8	Total Volume	50	1200

Table36

	A	B	C	D
1	Steps	Temperature (℃)	Time	Cycle
2	Initial denaturation	95	3 mins	1
3	Denaturation	95	15 seconds	35
4	Annealing	53	15 seconds	35
5	Extension	72	1 min	35
6	Final extention	72	5 mins	1

2. Gel electrophoresis